

Cloning, sequencing, and expression of Arthrobacter protophormiae endo-beta-N-acetylglucosaminidase in Escherichia coli.

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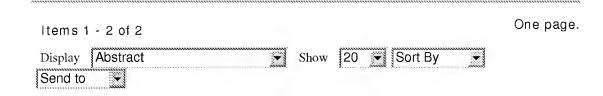
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The gene encoding endo-beta-N-acetylglucosaminidase from Arthrobacter protophormiae (Endo-A) was cloned, and its nucleotide sequence was determined. A single open reading frame consisting of 1935 base pairs and encoding a polypeptide composed of signal peptides of 24 amino acids and a mature protein of 621 amino acids was found. The primary structure of Endo-A exhibited significant homology with FO1F.10 gene product from Caenorhabditis elegans and weak homology with peptide-N4-(N-acetyl-beta-D-glucosaminyl)asparagine amidase from Flavobacterium meningosepticum and chitinase from Streptomyces olivaceoviridis. However, the enzyme had no significant homology with any previously reported endo-beta-Nacetylglucosaminidases. Transformed Escherichia coli cells carrying the 4.5-kb fragment expressed Endo-A activity. This enzyme activity was detected in the medium as well as in the periplasmic space of cells under the control of the Endo-A gene promoter. The recombinant Endo-A was efficiently isolated from the periplasmic space of the cells. N-terminal sequence analysis revealed that native and recombinant Endo-A have the same N-terminus. Recombinant and native Endo-A also showed very similar optimum pH profiles and transglycosylation activity.

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